Light Activation Mechanisms of Regulation of Photosynthesis in Bacteria

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Summary

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Many organisms utilize photoreceptor proteins to sense their environment, enabling them to detect advantageous or harmful conditions and to avoid dangers. This thesis aims to reveal the photochemistry of these receptor proteins, processes that occur on very short time scales, typically between 10^{-13} s and 10^{-9} s. For this purpose, time-resolved spectroscopy techniques are used. These techniques are specialized tools to observe and to follow the path of a chemical reaction of a protein that can be triggered by light. Understanding of the photo-dynamics of these photoreceptors provides an understanding of how light absorption is efficiently coupled to biological sensory function and opens up the possibility to alter the protein properties, such as the brightness of fluorescence, the emission/excitation wavelengths and the photo-stability.

In **chapter 2**, the AppA BLUF domain from *R. sphaeroides* that absorbs blue light through a noncovalently bound FAD chromophore, was investigated. Upon blue light absorption, the AppA BLUF domain forms a signaling state referred to as $AppA_{RED}$. Ultrafast transient absorption experiments showed that in contrast to many other photoreceptor proteins, the AppA BLUF domain is not photoreversible and does not enter alternative reaction pathways upon absorption of a second photon. To explain these properties, we propose that a molecular configuration is formed upon excitation of $AppA_{RED}$ that corresponds to a forward reaction intermediate previously identified for the dark-state BLUF photoreaction. Upon excitation of $AppA_{RED}$, the BLUF domain therefore enters its forward reaction coordinate, readily re-forming the $AppA_{RED}$ ground state and suppressing reverse or side reactions. Tryptophan fluorescence experiments showed that the conserved Trp-104 in the AppA BLUF remains buried in the protein matrix in both the dark and light states. This observation challenges the concerted Trp-104 – Met-106 'flip' model for AppA light activation that was proposed on the basis of BLUF crystal structures.

In **chapter 3** to **chapter 5**, the red light photoreceptors, bacteriophytochromes (Bphs) RpBphP2 (P2) and RpBphP3 (P3) from *Rp. palustris*, are studied. Bphs have two meta-stable states associated with a Z-anti and E-anti conformation of the C15=C16 methine bridge adjacent to ring D of their linear tetrapyrrole chromophore. These states are photo-switchable. P2 PAS – GAF – PHY and P3 PAS – GAF – PHY have a common Pr ground state but differ in their light-activated states, with P2 PAS – GAF – PHY forming a classical Pfr state absorbing at 750 nm, and P3 PAS – GAF – PHY forming a Pnr state absorbing at 650 nm. These differences presented us an

opportunity to elucidate the primary photochemistry of phytochromes that have been under extensive studies for the past few decades.

In **chapter 3**, investigation of P2 PAS – GAF – PHY and P3 PAS – GAF – PHY using ultrafast time-resolved visible absorption and emission techniques has allowed us to identify the factors that determine the fluorescence and isomerization quantum yields of these Bphs. P3 showed a fluorescence quantum yield that was significantly higher than that of P2. The excited state lifetime of the biliverdin chromophore in P3 was significantly longer than in P2 and other classical phytochromes, and was accompanied by a significantly reduced isomerization quantum yield. H/D exchange reduced the rate of decay from the excited state of biliverdin by a factor of 1.4 and increased the isomerization quantum yield. Comparison of the properties of the P2 and P3 variants showed that in P3, the quantum yields of fluorescence and isomerization are determined by excited-state deprotonation of biliverdin at the pyrrole rings, in competition with hydrogen-bond rupture between the D-ring and the apoprotein. With our kinetic model and the Bph X-ray structures at hand, we proposed solutions for engineering Bph into a highly fluorescent deep tissue fluorescence probe.

In **chapter 4**, our investigations on Bph were extended to Bph variants that lacked the PHY domain (PAS – GAF) or had a highly conserved Asp replaced by Ala. We showed an increase of the excited-state lifetime in the PAS – GAF constructs and the Asp mutants, which is probably related with a decreased excited-state deprotonation rate at the pyrrole rings. These results support the reaction model formulated in Ch. 3. The study suggested that a 40 ps component associated with a relaxation process of the excited state of P3 PAS – GAF – PHY represents local hydrogen-bond dynamics near the BV pyrrole nitrogens. We found that the PHY domain and the conserved Asp are essential to the formation of a canonical primary photoproduct Lumi-R. Finally, we demonstrated that the excited-state spectral properties of the Bphs are influenced by the hydrogen bonding partner(s) to ring D of the chromophore.

In **chapter 5**, an ultrafast mid-IR spectroscopy technique was employed to study the primary photochemistry of Bphs. This work has led to identification of a primary photoproduct band arising at ~1540 cm^{-1} that was not observed previously. By combining ultrafast mid-IR spectroscopy with FTIR spectroscopy on P2 and P3 wild type and mutant proteins, we demonstrated that the hydrogen-bond strength at the ring D carbonyl of the bilin chromophore is significantly stronger in RpBphP3 as compared to RpBphP2. This result is consistent with the X-ray structures of bacteriophytochrome, which indicate one hydrogen bond from a conserved histidine to the ring D carbonyl for classical bacteriophytochrome, and one or two additional hydrogen bonds from a serine and a lysine side chain to the ring D carbonyl for RpBphP3.